Evaluation of Bt Corn and RNA Interference Technology for Management of Larval Corn Rootworm in Central

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The purpose of this study was to evaluate the effectiveness of two Bt rootworm traits (Cry3Bb1 and Cry34/35Ab1) with and without an RNA interference trait (DvSnf7) for management of corn rootworm (CRW) larvae. The two CRW trait packages evaluated in this study were: 1) a pyramid Cry3Bb1 and Cry34/35Ab1 (SmartStax®), and a pyramid of Cry3Bb1, Cry34/35Ab1 and DvSnf7 (SmartStax PRO®). Both of the CRW trait packages were replicated twice, using two different hybrids with each trait package. Additionally, two non-CRW Bt hybrids were included in this study as untreated checks.

Materials and Methods

Study location. The study was conducted at the Iowa State University Johnson Farm. The field site had been planted the previous year with a trap crop, which is a mixed-maturity blend with a greater proportion of late-maturing varieties. This trap crop constitutes a favorable environment for adult female rootworm late in the season when other fields are maturing, and results in a high abundance of rootworm larvae the following year.

Field plot design. This study was a split-plot design with four replications. Treatments were four rows wide, and 35 feet in length. Plots were cut down to 30 feet in length after planting.

Planting. This study was planted on April 30 using a four-row John Deere Max Emerge™ 7100 Integral Rigid Frame Planter with 30 inch row spacing. We planted this study at a depth of two inches, with a spacing of six inches between seeds (35,600 seeds per acre). All treatments in this study consisted of pure stands of either rootworm traited corn or non-CRW Bt corn, with the exception Treatment 4: Cry3Bb1 & Cry34/35Ab1, which contained a blend of 95% CRW Bt corn and 5% non-CRW Bt corn. All seed in this study contained Bt traits for aboveground pests (Cry1A.105 and Cry2Ab2).

Stand counts. On June 1, early season stand counts were measured in all treatments. These were measured by using a 2-inch PVC pipe cut to the length of 17.4 feet (1/1,000 of an acre for 30-inch row spacing) that was placed between two rows of corn and the number of plants in both rows then counted. Late season stand counts were measured on October 4 following the same procedure as early season stand counts. Measurements for both dates were averaged to provide a single value for stand counts (Table 1).

Table 1. Stand Counts.

Treatment[b]	Stand Counts[c,d]
Treatment 5: Cry3Bb1, Cry 34/35Ab1, & DvSnf7	31.6
Treatment 6: Cry3Bb1, Cry 34/35Ab1, & DvSnf7	30.7
Treatment 4: Cry3Bb1 & Cry 34/35Ab1*	30.5*
Treatment 1: Non-CRW Bt	30.1
Treatment 2: Non-CRW Bt	30.0
Treatment 3: Cry3Bb1 & Cry 34/35Ab1	29.4

[a]Planted April 30, 2021; evaluated June 1 and October 4, 2021.
[b]Non-CRW Bt = an absence of any Bt trait targeting CRW.
[c]Data presented as plants per 1/1000 of an acre.
[d]No significant differences between means (ANOVA, $P \geq 0.05$).
*This treatment contained a blend of 95% CRW BT corn and 5% non-CRW Bt corn.

Root injury. After the majority of CRW larvae had finished feeding on corn roots, roots were dug to assess feeding injury. Plants from Treatment 4: Cry3Bb1 & Cry34/35Ab1 were gene checked prior to digging roots to ensure no non-CRW Bt plants were evaluated for root injury. Roots were dug on August 10. Prior to leaving the field, all roots were labeled with study name and plot number using a permanent marker. Roots were cleaned at the ISU Johnson Farm's root washing station. Roots were first soaked in water for 2-8 hours and then washed with a hose to remove any remaining soil. Roots were evaluated on August 13 for rootworm feeding injury following the Iowa State Node Injury Scale (0-3) (Table 2).

Node Injury Scale (0-3)

0.00: no feeding injury (lowest rating that can be given).

1.00: one node (circle of roots), or the equivalent of an entire node, pruned to within 1.5 inches of the stalk or soil line.

2.00: two nodes pruned.

3.00: three or more nodes pruned (Highest rating that can be given).

Injury in between complete nodes pruned was quantified as the proportion of the node missing (1.50=one and a half nodes pruned).

Product consistency. Percent product consistency was calculated as the percentage of times a treatment limited feeding injury to 0.25 nodes or less (greater injury may result in economic yield loss, especially when plants are moisture stressed).

Yields. This study was machine harvested on October 20 with a modified John Deere 9450 plot combine owned by Iowa State University. Weight (pounds) and percent moisture were recorded with a high-capacity grain gauge, using HarvestMaster brand harvest data collection system. These measurements were converted to bushels per acre of No. 2 shelled corn (56 pounds per bushel at 15.5% moisture) in Table 3.

Data analysis. Data were analyzed with analysis of variance (ANOVA) in SAS Enterprise Guide 7.1. The treatment means were compared using LSMEAN procedure with an experiment wise error rate of $P < 0.05$.

Table 2. Root Injury and Product Consistency.

Treatment[b]	Node-Injury[c,d,f]	Product Consistency,[f]
Treatment 6: Cry3Bb1, Cry 34/35Ab1, and DvSnf7	0.15a	78a
Treatment 5: Cry3Bb1, Cry 34/35Ab1, and DvSnf7	0.18a	75a
Treatment 4: Cry3Bb1 and Cry 34/35Ab1*	0.99b*	8b*
Treatment 3: Cry3Bb1 and Cry 34/35Ab1	1.22c	8b
Treatment 2: Non-CRW Bt	2.19d	3b
Treatment 1: Non-CRW Bt	2.36d	0b

[a]Planted April 30, 2021; evaluated August 13, 2021.
[b]Non-CRW Bt = an absence of any Bt trait targeting CRW; All treatments contained traits for above ground pests.
[c]Means based on 40 observations (10 roots/treatment x 4 reeplications).
[d]Iowa State Node-Injury scale (0-3). Number of full or partial nodes completely eaten.
[e]Product consistency = Percentage of times nodal injury was 0.25 (¼ node eaten) or less.
[f]Significant difference between the treatment means for both Node-Injury and Product Consistency (ANOVA, $P < 0.05$).
*This treatment contained a blend of 95% CRW BT corn and 5% non-CRW Bt corn, however, gene checks were used prior to rating roots to ensure that only CRW Bt plants were evaluated for root injury.

Table 3. Average Yield. Johnson Farm, Story Co.[a]

Treatment[b]	Bushels per Acre[c,d,e]
Treatment 6: Cry3Bb1, Cry 34/35Ab1, & DvSnf7	220.5[a]
Treatment 5: Cry3Bb1, Cry 34/35Ab1, & DvSnf7	212.9[a]
Treatment 3: Cry3Bb1 & Cry 34/35Ab1	193.5[b]
Treatment 4: Cry3Bb1 & Cry 34/35Ab1*	174.0[c]*
Treatment 2: Non-CRW Bt	144.9[d]
Treatment 1: Non-CRW Bt	144.3[d]

[a]Planted April 30, 2021; harvested October 20, 2021.
[b]Non-CRW Bt = an absence of any Bt trait targeting CRW.
[c]Means based on 4 observations (4-row treatment x 30 row-feet/treatment x 4 replications).
[d]Significant differences between means (ANOVA, $P < 0.05$).
[e]Yields converted to 15.5% Moisture.
*This treatment contained a blend of 95% CRW BT corn and 5% non-CRW Bt corn.

Results and Discussion

Feeding pressure from larval corn rootworm at this location was very high, with the untreated checks suffering over two nodes of root injury (Table 1). It is noteworthy that Bt corn with a pyramid of Cry3Bb1 and Cry34/35Ab1 suffered an average of a node or more of feeding injury (Table 1). The US EPA defines more than half a node of rootworm injury to pyramided corn as greater than expected injury, and is a reason to suspect the presence of Bt-resistant rootworm. In Iowa, western corn rootworm populations have been identified with resistance to all Bt traits. It is highly likely that the pronounced injury to corn with a pyramid of Cry3Bb1 and Cry34/35Ab1, observed in this study, resulted from the presence of western corn rootworm with resistance to both of these Bt traits. The presence of the novel insecticidal trait DvSnf7 reduced average root injury to less than 0.25 nodes, and provided adequate root protection against this rootworm population (Table 1).

Patterns of product consistency and yield closely mirrored root injury (Tables 1 and 3). Non-Bt corn had the lowest product consistency and yield, pyramided Bt corn displayed an intermediate level of product consistency and yield, and these metrics were highest for corn that contained both DvSnf7 and Bt traits. Because stand counts were similar among treatments, it is likely that differences in yield can be attributed primarily to differences in root injury (Table 2).

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Additional Information

Annual reports for the Iowa Evaluation of Insecticides and Plant-Incorporated Protectants (ent.iastate.edu/dept/faculty/gassmann/rootworm) are available online through the Department of Entomology at Iowa State University.